The distribution, within the eggshell, of the pigments producing egg coloration

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INTRODUCTION

This paper consists of a short description of the normal distribution of pigments in and on the eggshell, and the way in which they govern the apparent external coloration of it. Most of this was discovered by Von Nathusius in the late nineteenth century. His work was largely overlooked by later writers although it has recently been brought together, translated, and editied by C. Tyler (pers. comm.). In view of the published information by Schönwetter (1927), Hobson (1947), Pring (1958-62) and Friedmann (1948), which appears to indicate some degree of confusion regarding egg pigmentation, it seemed advisable to set out this description of pigment distribution.

The information given below is based on data gained by microscopic examination of radial sections of eggshell, and by the grinding away of surface layers. The facts given explain the coloration of the great majority of birds' eggs. There are exceptions, but it is proposed to make a more detailed study of these, and they are not described here.

SHELL STRUCTURE

The shell of a bird's egg is generally regarded as consisting of three layers. The innermost layer rests on the shell membrane and is well supplied with air-spaces. The shell is formed on a base consisting of a mass of separate nodules deposited on the shell membrane with a network of spaces between them. The remainder of the shell is built up with these nodules as a base, the air-spaces persisting and being visible in radial section as a series of small holes in the shell structure immediately above the membrane. In some eggs, particularly those of the Galliformes and Falconiformes these nodules appear as a series of rounded projections from the inner surface of the shell, giving this layer the name by which it is generally known, the Mammillary Layer.

Immediately above this is the main thickness of the shell. This, like the mammillary layer, consists of a hard crystalline material laid down on a basis of protein fibres. Owing to the hardness of the shell, portions of it were, in the past, normally decalcified before microscopic examination was possible, and the crystalline structure, mainly calcium carbonate, was lost. As a result this portion of the shell appeared as a fibrous mass, and was given the inappropriate name of the Spongy Layer. The outermost layer of the shell is known as the Cuticle. This is normally regarded as a thin transparent outer coating, but recent work by Tyler (in press, and pers. comm.) has shown that this layer may vary considerably in thickness, and in some families it is considerably thicker and far more complex in structure than was formerly believed.

PIGMENTATION

1. Pervasive or Ground Pigment

The mammillary and spongy layers may be one of two colours, either white or blue. There does not seem to be any definite evidence of a white pigment being present, and the white colour appears to be the result of reflection and refraction of light from internal surfaces in the crystalline structure. Where there are many such surfaces the material of the shell is very white, and broken surfaces have a rather granular appearance. Where there are few the material is more translucent and less white, while a broken surface will appear glassy. In general the spongy layer tends to appear whiter, and the mammillary layer less white and more translucent. In some families the spongy layer may be partly translucent, particularly towards the outer surface where there may be a transparent zone either immediately under, or perhaps as a part of, the cuticular layer. The apparent whiteness of the shell structure in the eggs of many species appears to coincide with the distribution of the protein matrix fibres as given by Romanoff and Romanoff (1949). Where such fibres are most numerous as, for example, in the inner part of the spongy layer, the shell appears whiter in radial section, and it is possible that these fibres may help to create the structural interference that results in the white appearance.

Where blue occurs as a shell colour it is normally present with uniform distribution throughout the thickness of the shell. Its mode of distribution is very even, without obvious variation, and its occurrence is due to the presence of a blue-green pigment, Biliverdin, (Lemberg 1934) this being the pigment which Sorby (1875) called Oöcyan. Where translucent or transparent zones are present in a blue shell they appear darker blue than the rest of the shell owing to the absence of white light reflected from internal surfaces. Within birds' eggs as a whole it is possible to find a complete gradation from deep blue to pure white. In the White-browed Jay-thrush, *Garrulax sannio*, the difference is individual, different birds laying either white or blue eggs; and in some other passerine species where egg colour is variable, patterned shell may show a ground colour which is either white or blue in different individuals; while white eggs may occur abnormally in species which normally lay blue eggs.

2. Pattern Pigment

The patterns on eggshells appear to be produced by a single pigment which may appear as three different colours, red, brown, or black, or intermediate shades of these. The pigment in normally applied to the surface of the egg in separate masses which form spots or blotches on it. In some species such as the Quail, *Coturnix coturnix*, it is purely superficial, but in the majority of birds with patterned eggshells it is applied at intervals during the period of shell formation. If shells of the latter type are examined in radial section the pigment will be seen to occur as dark layers parallel to the surface of the shell, and may be at any depth within the thickness of the spongy layer although tending to be more frequent towards the outside. Such pigment layers may occur one above the other.

The shell itself is frequently translucent, and colour may show through it. In freshly laid white eggs of smaller birds it is sometimes possible to see the colour of the yolk showing through the shell. Where spots of pigment are present within the thickness of the shell they are frequently apparent on the outside of it, although this depends to some extent on their depth within the shell. Their appearance is modified by the thickness of the white or blue spongy layer overlaying them, and they will appear paler and slightly different in colour, being grey, purple, lilac, pink, fawn, or similar shades. Their appearance may also vary according to the thickness and depth of colour of the pigment layer which is present. In some cases this pigment may be present over the whole surface or throughout the shell, either as minute specks or generally diffused. The shell may then appear wholly grey, red, brown, or some paler shade of these.

3. Superficial Pigment

In many eggs there is a yellowish pigment present in or under the cuticular layer. This colour is usually associated with a thin transparent cuticular layer and modifies the appearance of underlying pigments. Where buff pigment occurs it is also only apparent as a superficial pigment layer. There seems insufficient evidence to show whether these buff and yellow pigments represent two different superficial pigments or whether they are forms of the same pigment. Some authorities appear to regard both the superficial and the pattern pigments as forms of a single red-brown pigment (Romanoff and Romanoff, *op. cit.*). In some eggs the superficial pigment appears to extend a little way into the spongy layer, but in view of Tyler's findings (in press) concerning the structure of the cuticular layer in the waterfowl it seems inadvisable to make assumptions as to where the cuticular layer ends and the spongy layer begins. As a result of the presence of these superficial pigments, white eggs may appear cream, yellow, or buff, while blue shells may appear some shade of green or olive. Removal of the cuticular layer by acid or abrasion will reveal the colour of the spongy layer. Most green eggshells, with one or two exceptions, are blue with a superficial yellowish layer. Such a layer will also modify the appearance of the pattern pigments to some extent, making them appear darker.

FINAL COMMENTS

From the above information it can be seen that the colour of the majority of eggshells is due to the presence of a very limited number of pigments. There may be a blue colour throughout the shell thickness; red, brown, or black markings may either be superficial or scattered through the thickness of the spongy layer; and a superficial yellow or buff layer may be present. The different colouring of eggshells is the result of the way in which the pigments present are arranged. A few examples will illustrate this. The yellow-and-black patterned eggs of the Red Grouse, *Lagopus scoticus*, have a white shell with a superficial layer of black pigment patches covered by a yellow cuticular layer. The red-spotted blue eggs of the Mistle Thrush, *Turdus viscivorus*, have a blue shell with patches of red-brown pigment distributed throughout the thickness of the spongy layer. The buff eggs, with reddish spots, of the Moorhen, *Gallinula chloropus*,

have a white shell with patches of reddish pigment distributed throughout the spongy layer and a superficial buff layer. The greenish, black-spotted eggs of the Carrion Crow, *Corvus corone*, have a blue shell with black pigment patches distributed throughout the thickness of the spongy layer, and a superficial yellowish layer.

The original descriptions of the eggs of some rare species have been based on eggs taken from the oviduct of a dead bird, and such eggs occur in collections. It should be remembered that an oviduct egg usually lacks a cuticular layer, and the colour shown by the shell is usually that of the spongy layer. In such circumstances buff eggs will appear white and green or olive eggs will appear blue.

The above descriptions give some indication of the way in which pigment distribution contributes to the final coloration of the eggshell. Much of this information is self-evident, and this account supplements earlier descriptions mainly with regard to the extent to which the blue colour and pattern pigments have been found to occur within the structure of the shell.

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